

A nonhuman primate model for aerosol infection with western equine encephalitis viruses

Douglas S. Reed, Thomas Larsen, Catherine
Wilhelmsen, William Pratt, Cathleen Lind, and
Michael Parker
USAMRIID

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals, National Research Council, 1996*. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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Western Equine Encephalitis Viruses (WEEV)

- Endemic in Western North America
- Naturally transmitted by mosquitoes; outbreaks are uncommon
- By mosquito bite, incapacitating illness in humans that is rarely fatal
- By aerosol, laboratory accidents suggest 40% mortality rate
- Rarely studied in nonhuman primate (NHP)
 - old reports from 1930s with rhesus

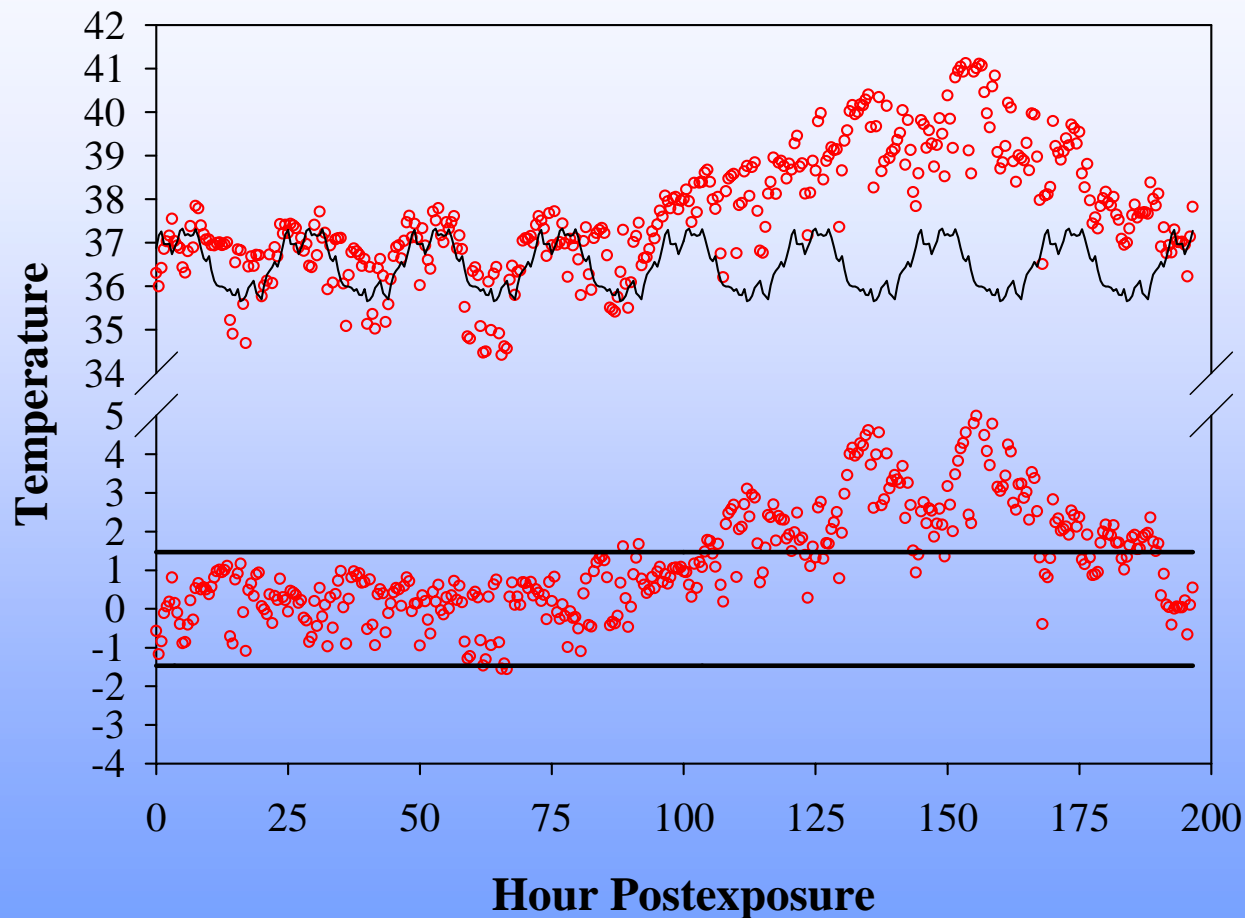


NHP Model for Aerosol Exposure to WEEV

- Species: *Macaca mulatta*, the rhesus macaque
Macaca fascicularis, the cynomolgus macaque
- Virus:
 - WEEV (CBA-87)
- Lethal model
 - Monitor physiological response by radiotelemetry
 - Temperature, heart rate, blood pressure
 - LD₅₀
 - Disease course after aerosol exposure
 - Viremia, CBC, clinical chemistries



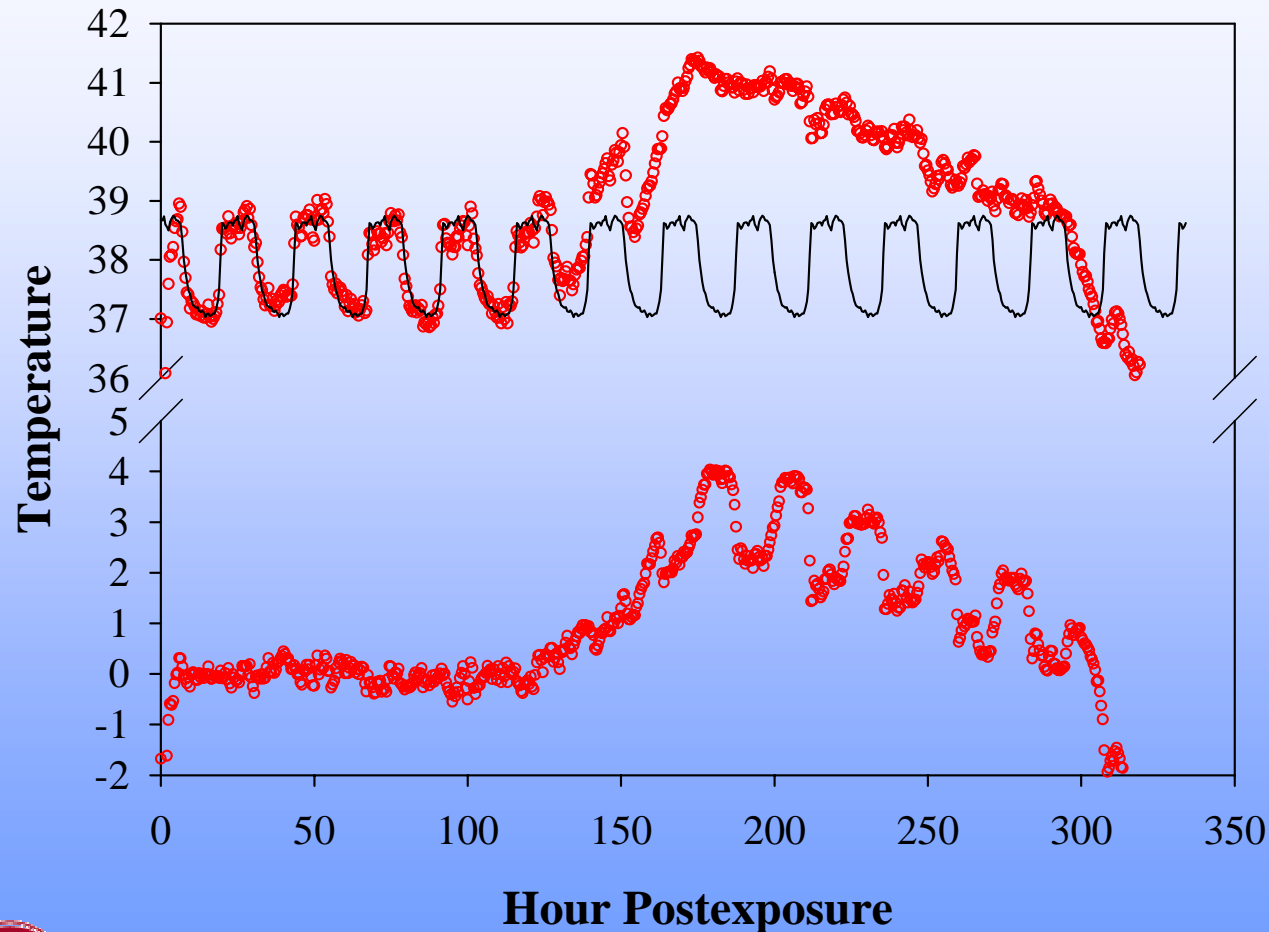
Fever Response After Aerosol Exposure to WEEV in a Rhesus Macaque



Tmax: 5.1°C
(41.1°C)
Duration: 72.5 hrs
Fever-hr: 197.5



Fever Response to Aerosol Exposure to WEEV in a Cynomolgus Macaque



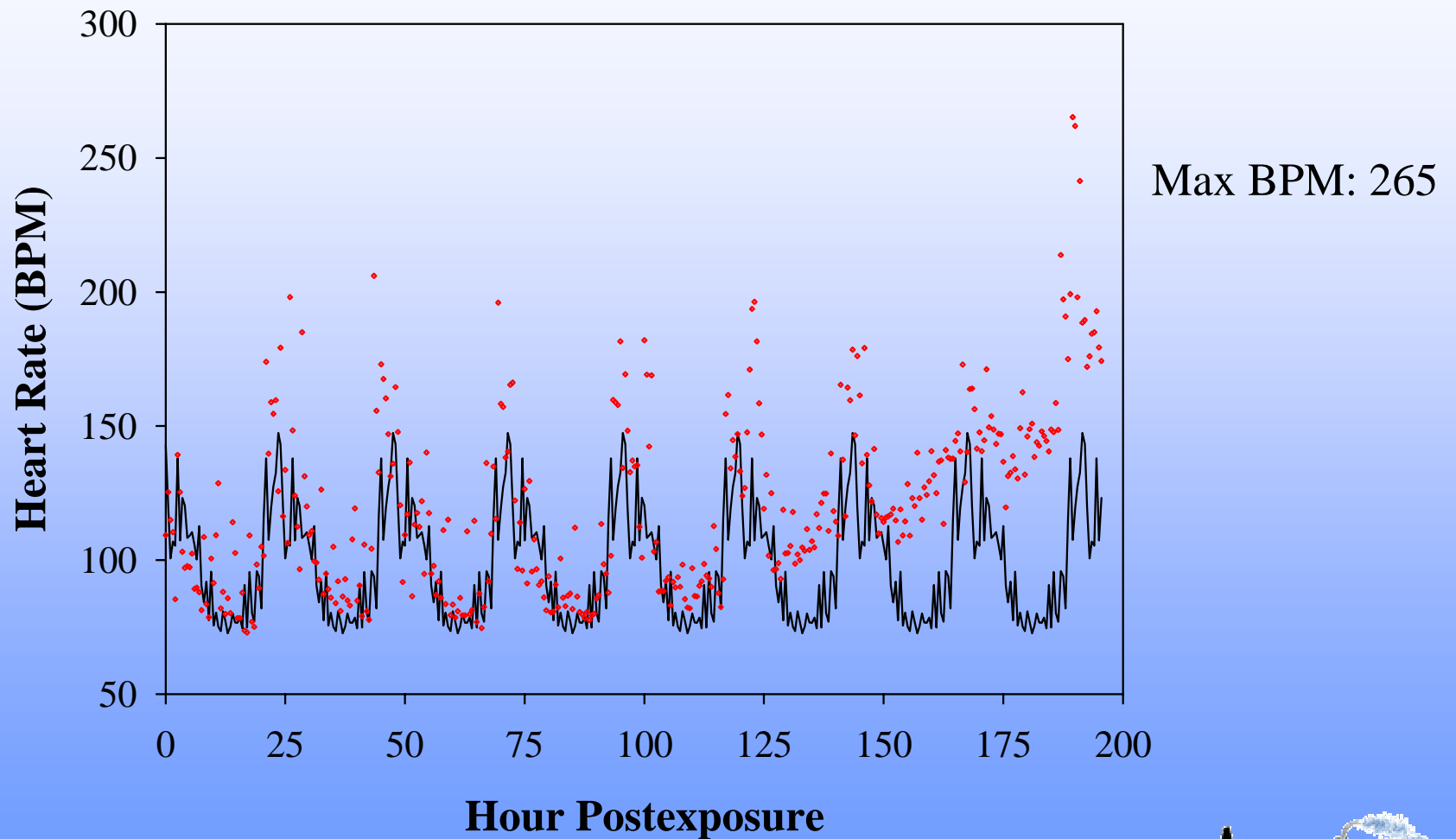
Tmax: 4.03°C
(41.4°C)
Duration: 169 hrs
Fever-hr: 326.3



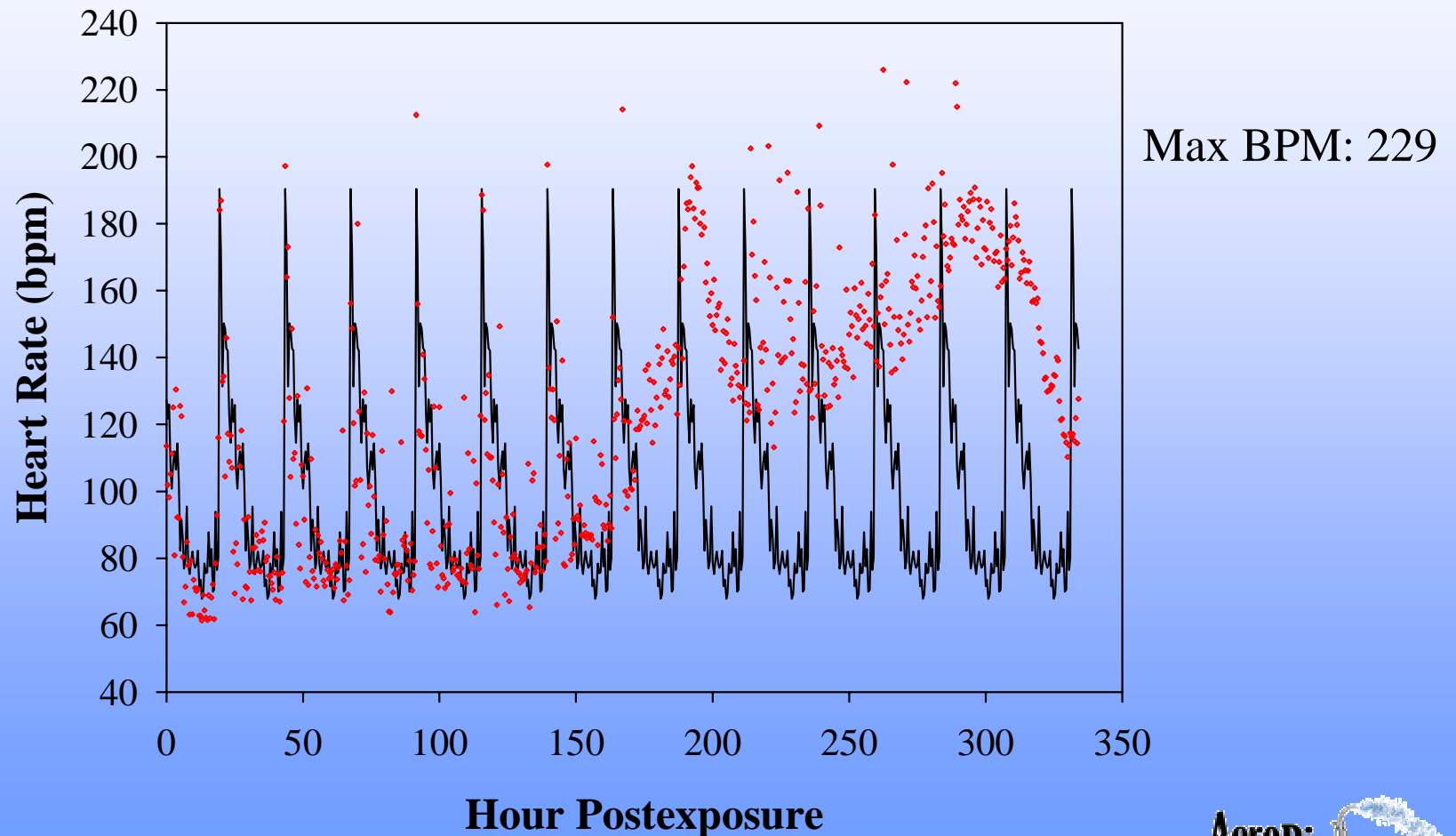
Inhaled dose: 4.5 log₁₀ pfu



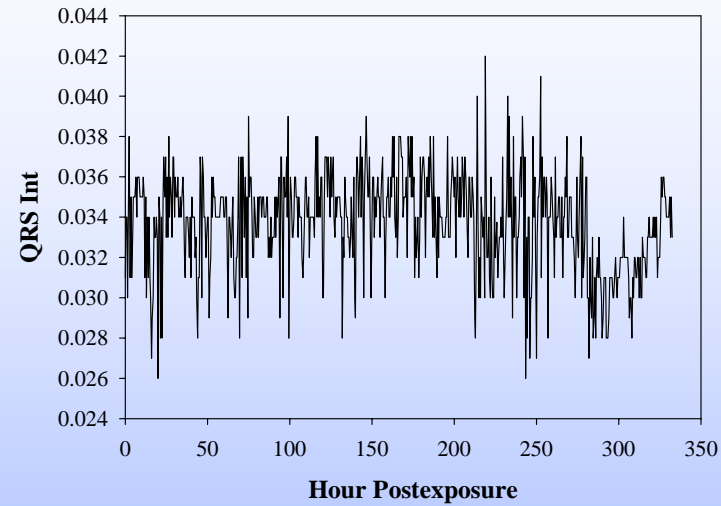
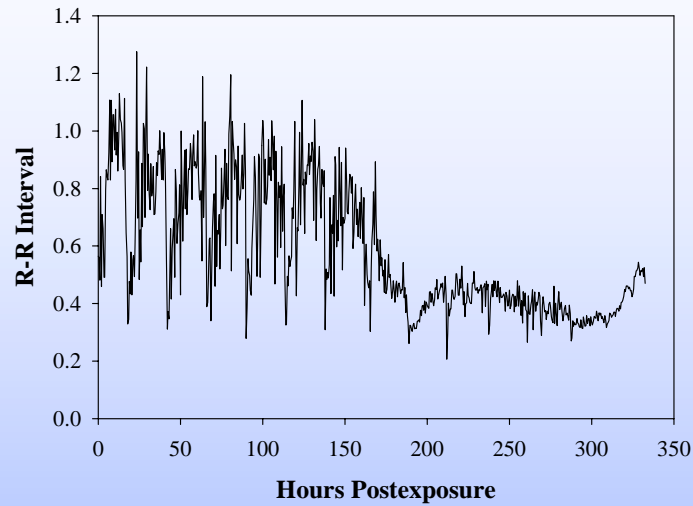
Heart Rate Increase After Aerosol Exposure to WEEV in a Rhesus Macaque



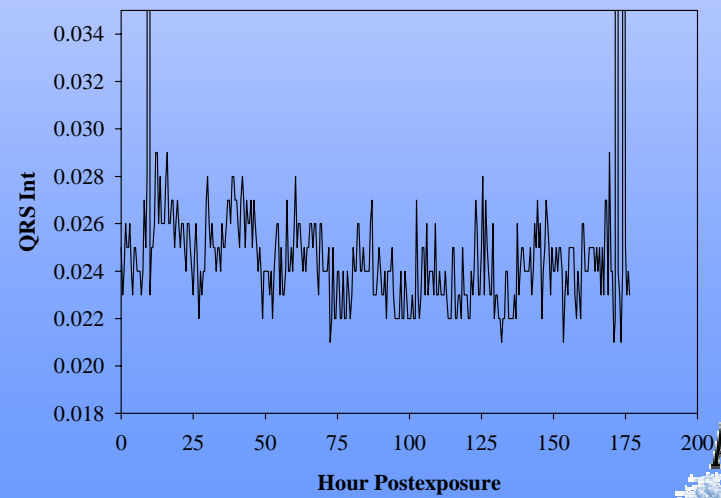
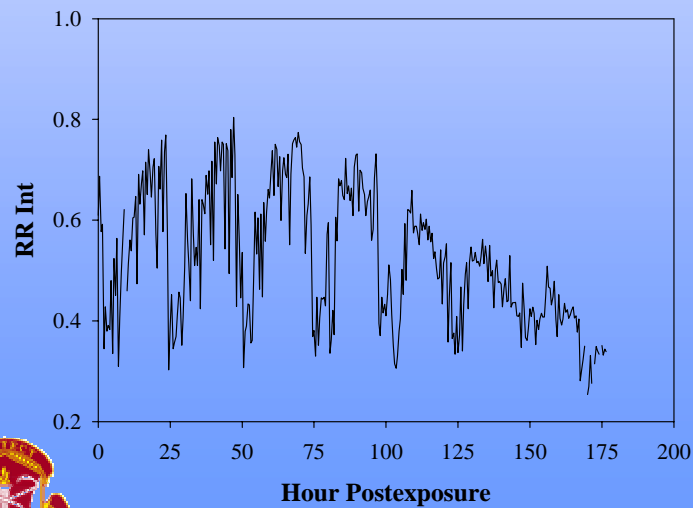
Heart Rate Increase After Aerosol Exposure to WEEV in a Cynomolgus Macaque



Analysis of ECG Data From Macaques



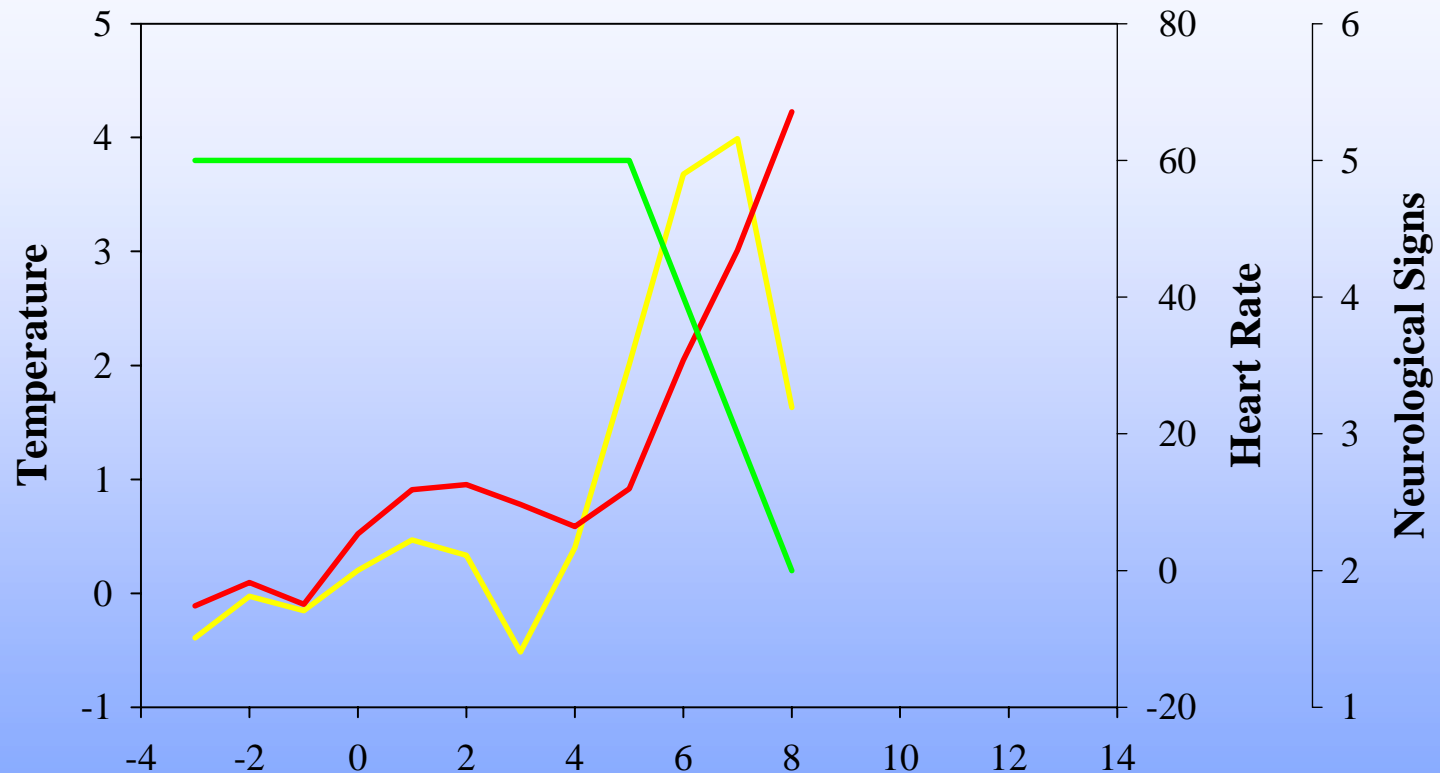
Cyno



Rhesus



Clinical Signs After Aerosol Exposure to WEEV in a Rhesus Macaque



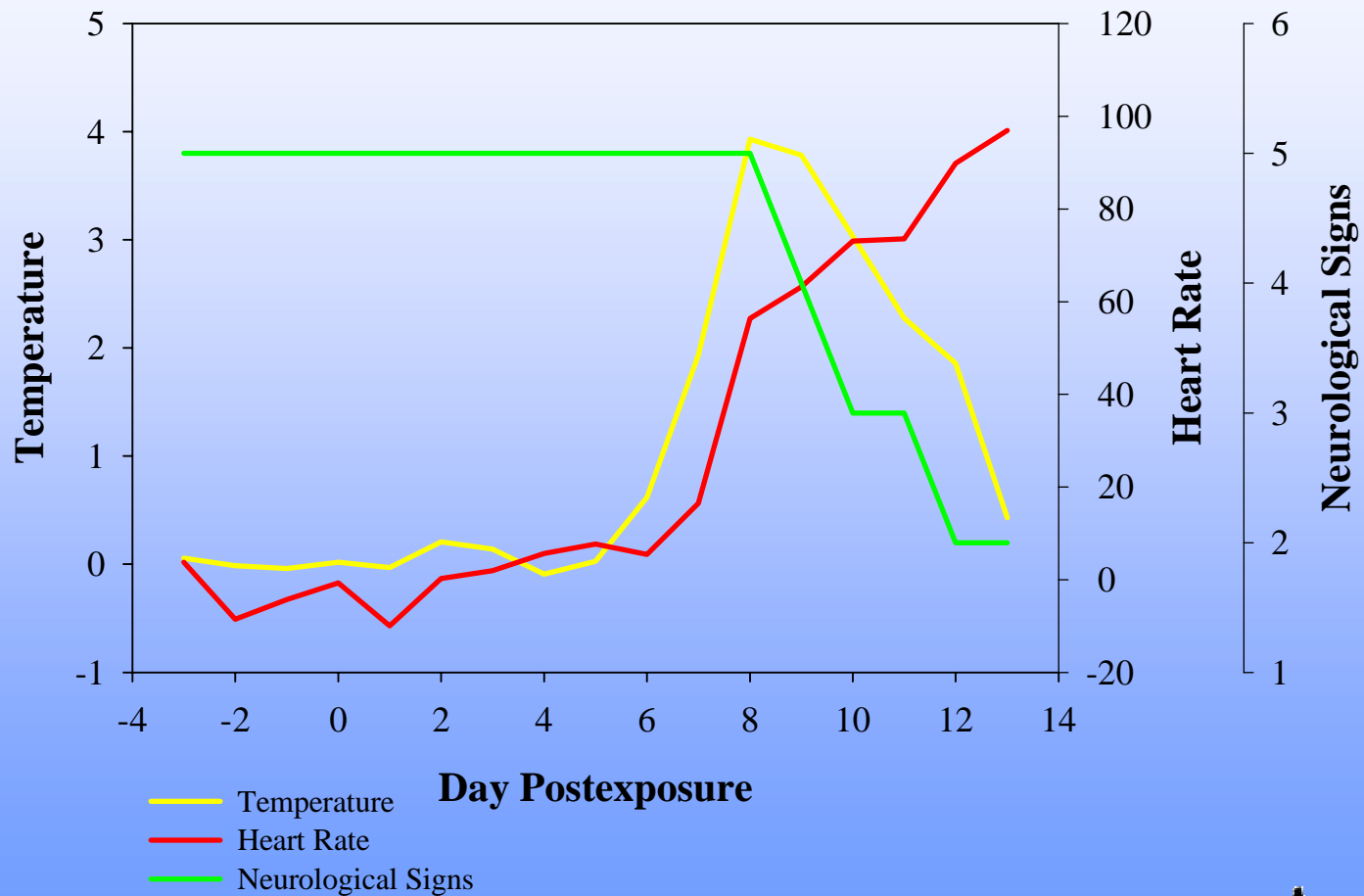
— Temperature
— Heart Rate
— Neurological Signs

Day Postexposure

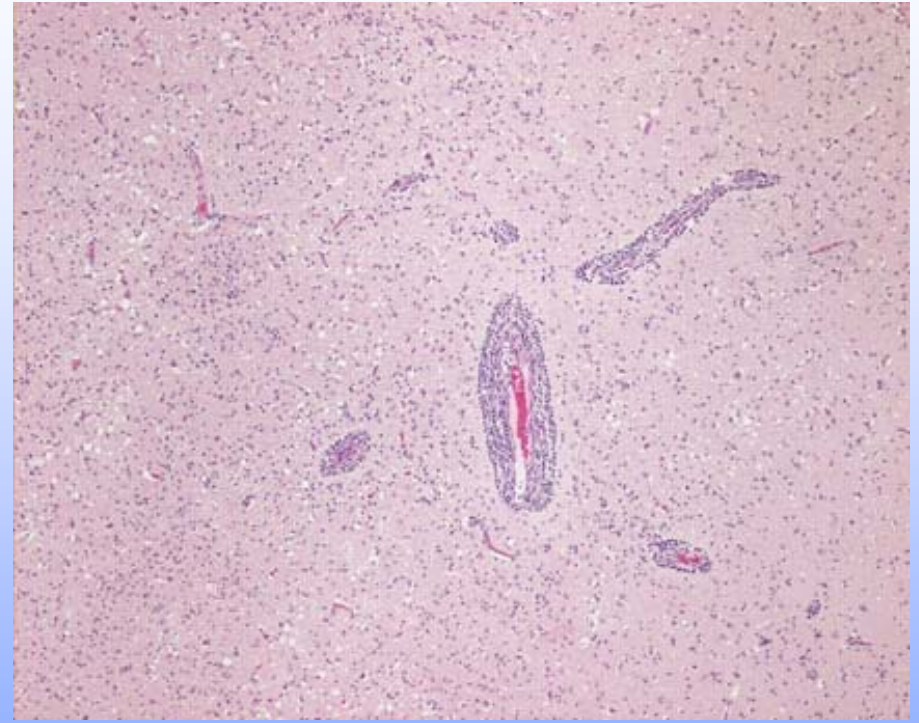
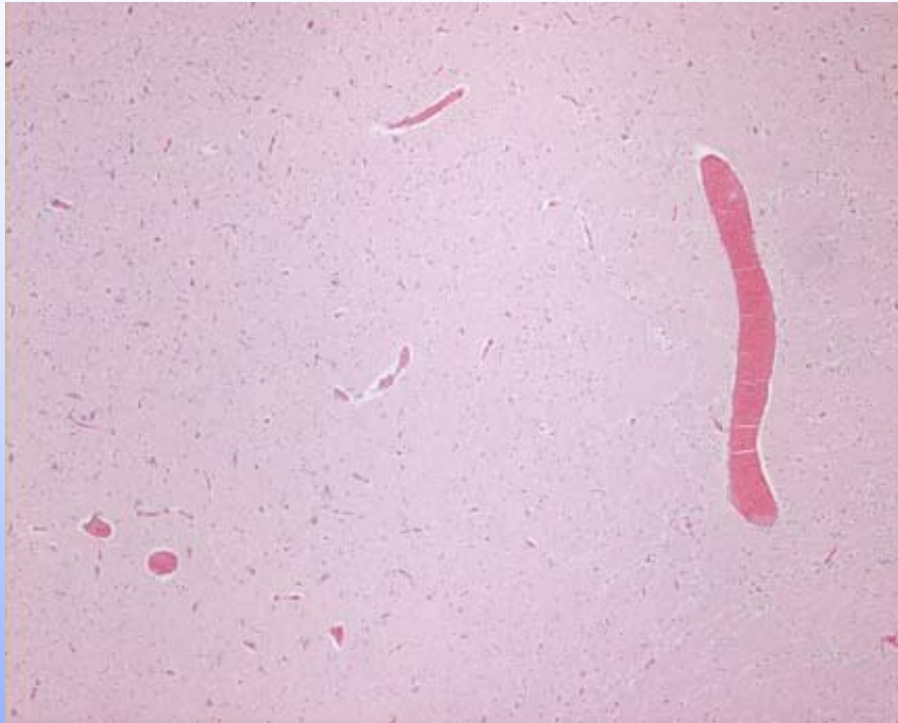
Neurological Signs
 5 = Normal
 4 = Depression
 3 = Occasional seizures
 2 = Frequent seizures
 1 = Comatose



Clinical Signs After Aerosol Exposure to WEEV in a Cynomolgus Macaque



WEEV Pathology



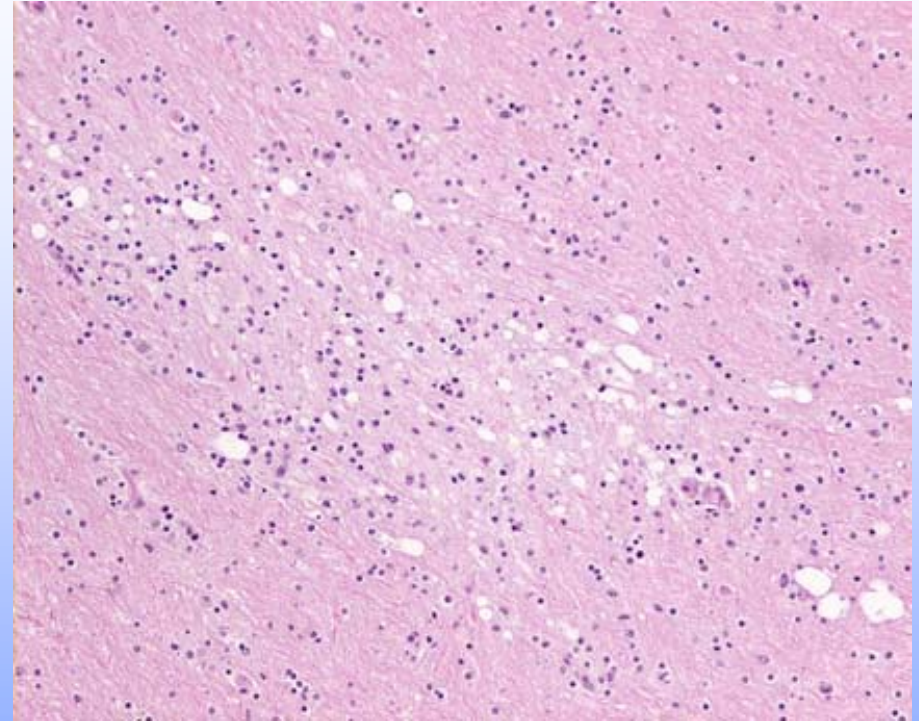
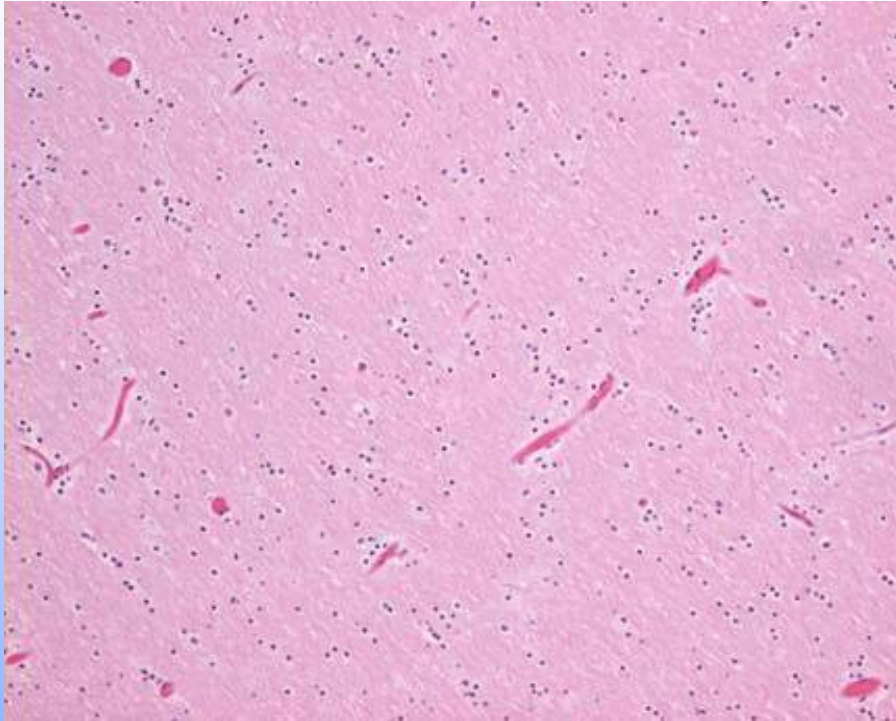
CNS, gray matter. Comparative slide demonstrating normal on the left and WEEV-infected tissue on the right. Note the increased cellularity on the infected animal, the marked perivascular cellular infiltrate, and vacuolization of the neuropil. Cynomolgus macaque, H&E, 10X.



Photo courtesy of Tom Larsen



WEEV Pathology



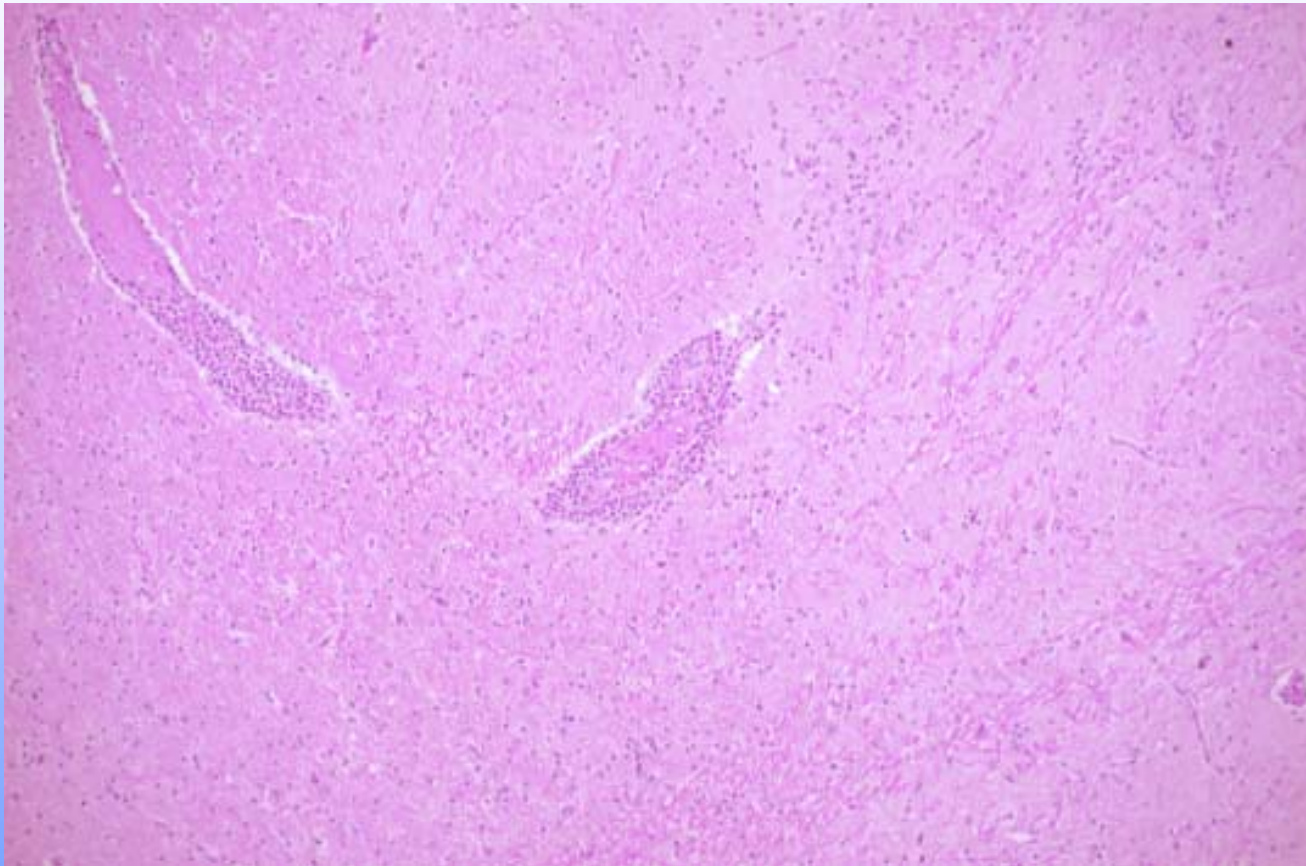
CNS, white matter. Comparative slide demonstrating normal on the left and WEE-infected tissue on the right. Note the increased cellularity, disorganization, and vacuolization (demyelination) in the infected animal. Cynomolgus macaque, H&E, 10X.



Photo courtesy of Tom Larsen



WEEV Pathology

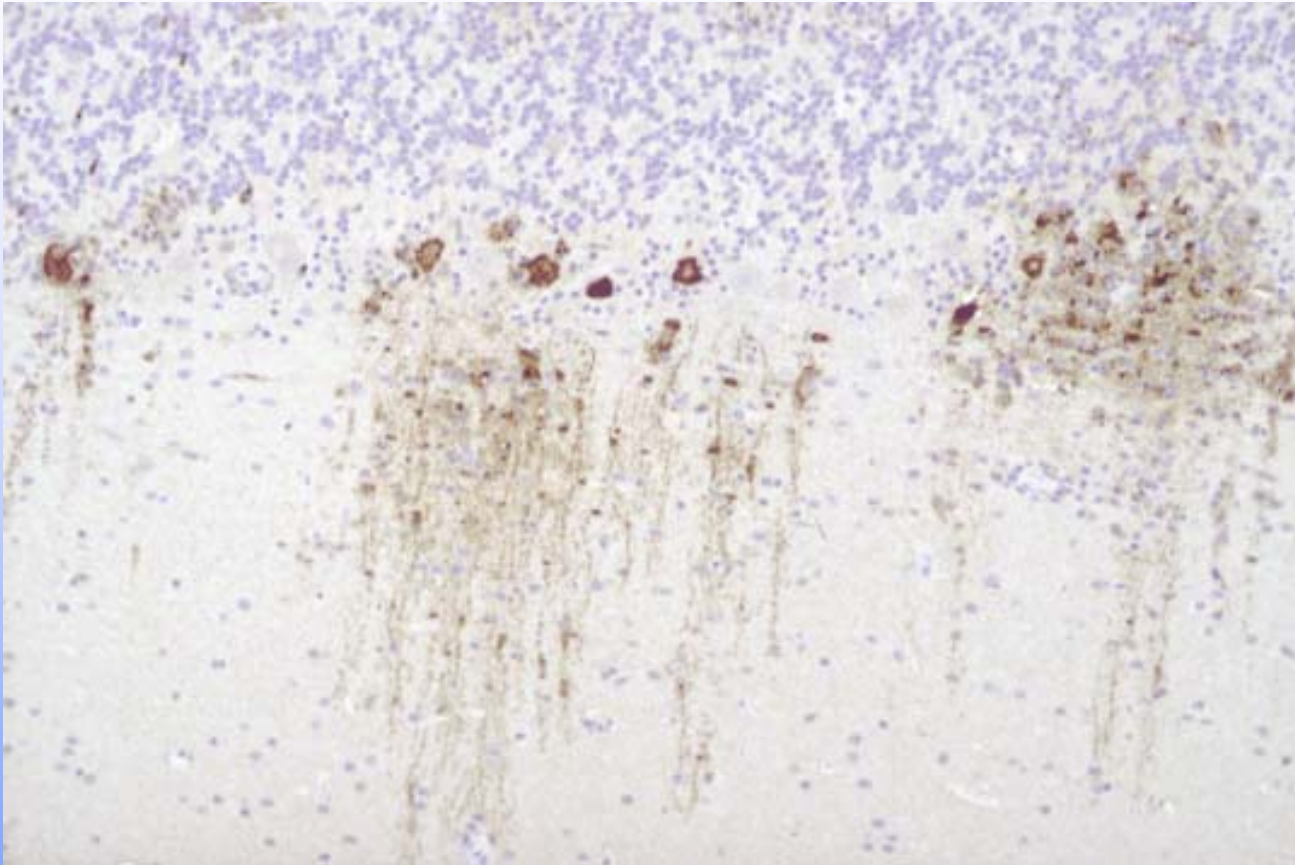


Rhesus brain, infiltrate, perivascular cuffs, H&E

Photo courtesy of Catherine Wilhelmsen



WEEV Pathology

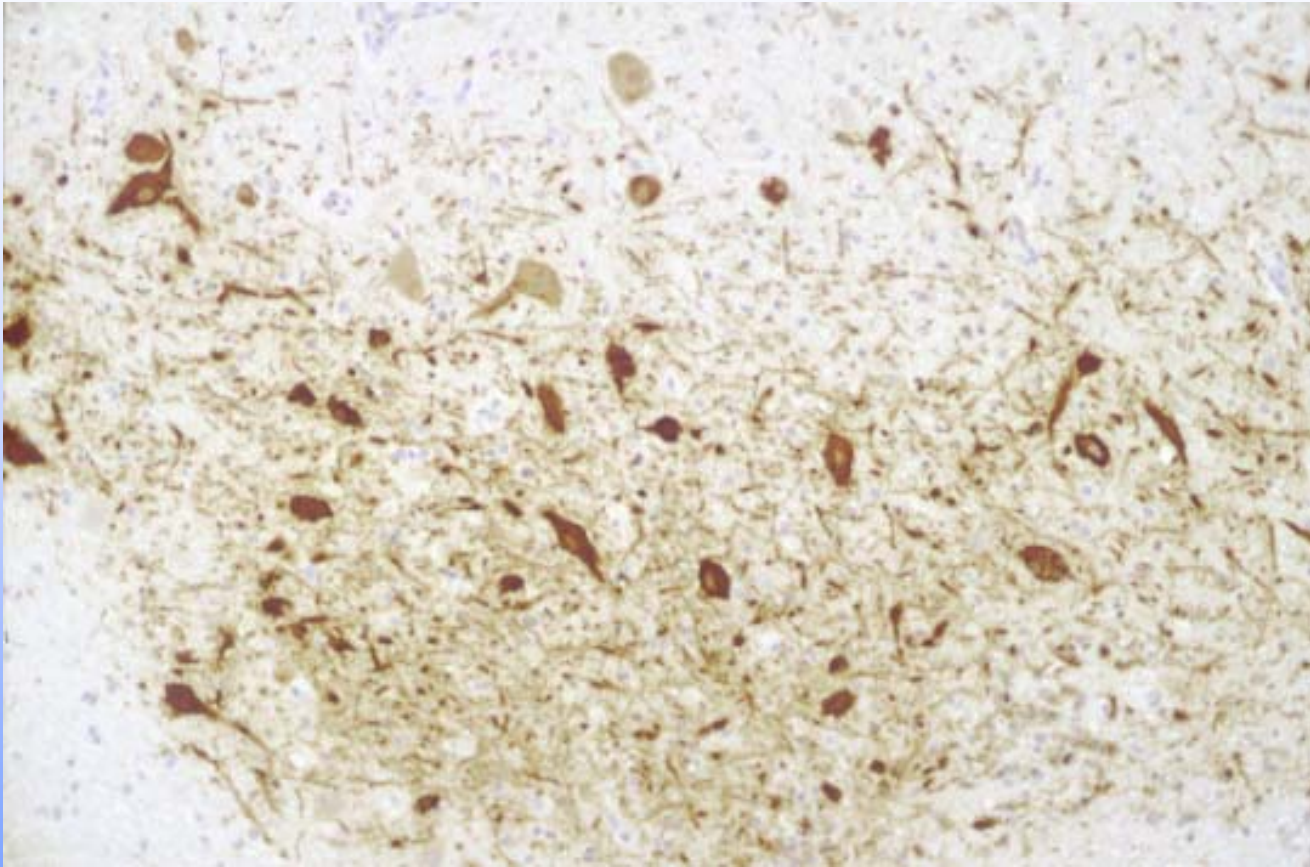


Viral antigen in Purkinje cells, cerebellum, rhesus macaque

Photo courtesy of Catherine Wilhelmsen



WEEV Pathology



Viral antigen in neurons of cerebellar peduncle, cerebellum, rhesus macaque



Photo courtesy of Catherine Wilhelmsen



WEEV Pathology

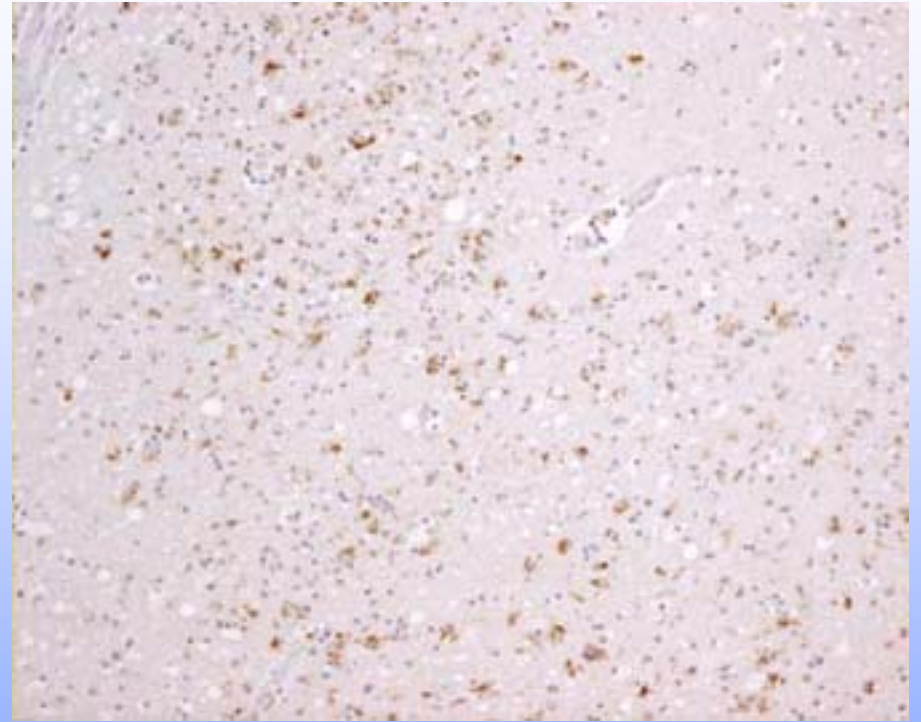
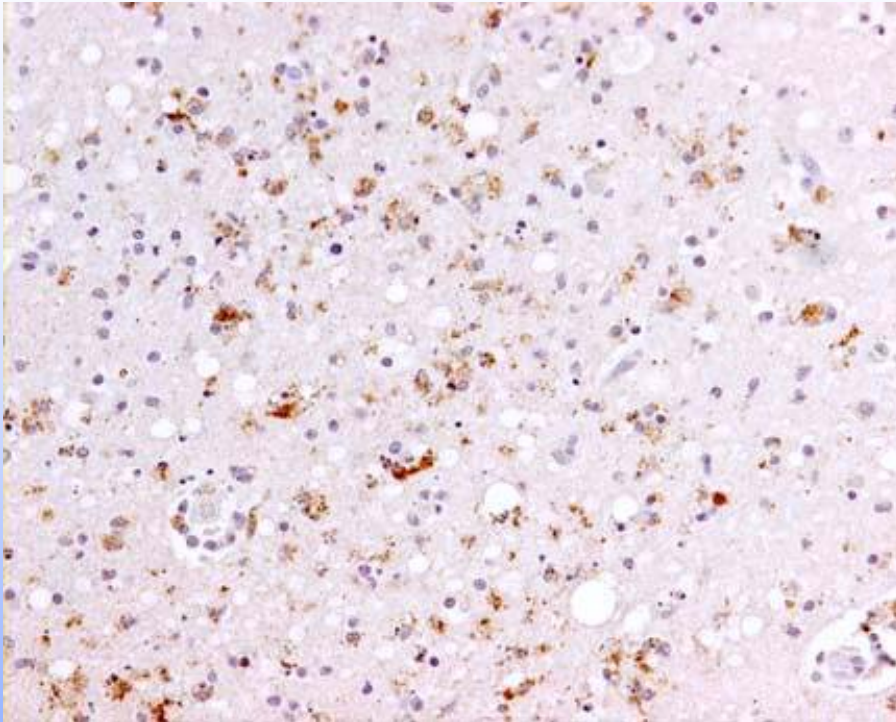


Viral antigen in pontine neurons, pons, rhesus macaque

Photo courtesy of Catherine Wilhelmsen



WEEV Pathology



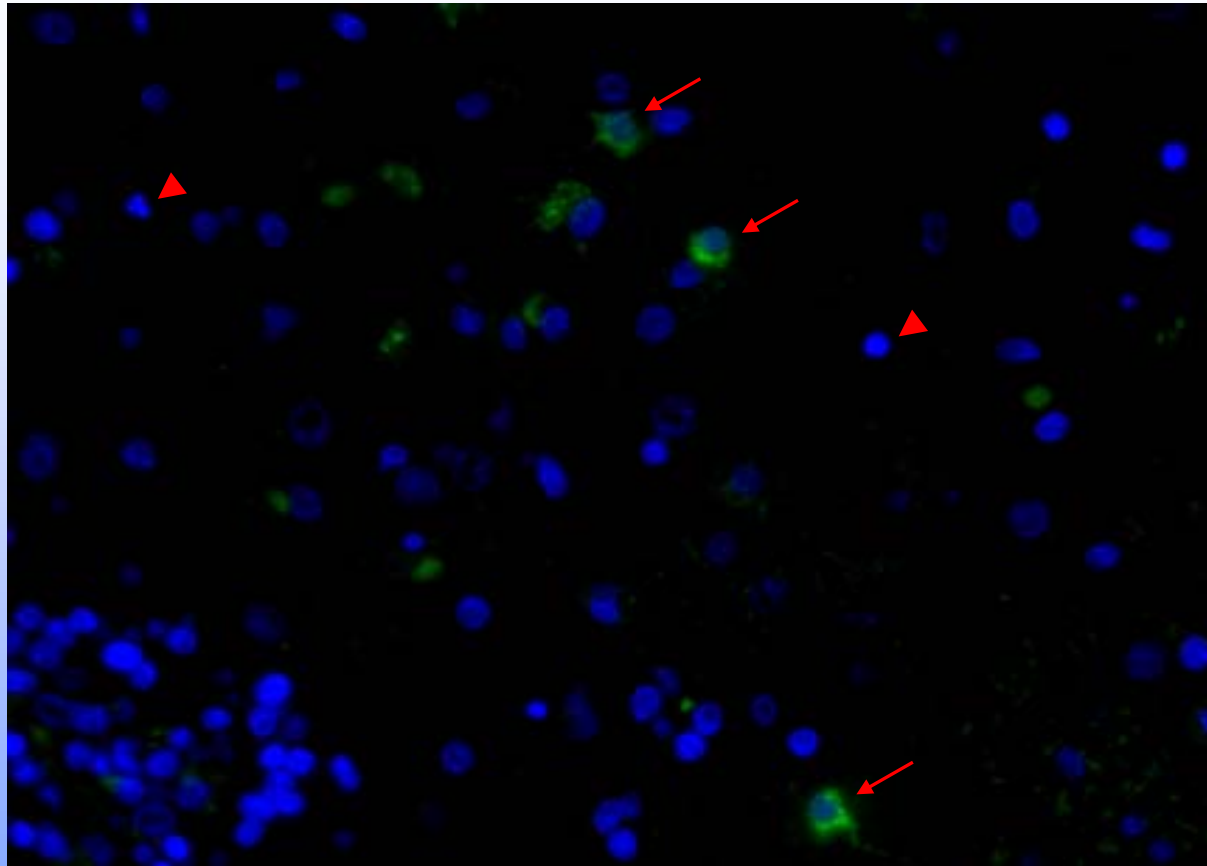
CNS, gray matter. Note the marked immunopositive tissue (brown staining) in this focal area of inflammation, increased cellularity of the neuropil, and apoptotic bodies. Immunoperoxidase stain, 10X & 4X.



Photo courtesy of Tom Larsen



WEEV Pathology



CNS, gray matter. Note the WEE-immunopositive neurons (arrow) and the apoptotic bodies (arrowhead) in this focal area of inflammation. Immunofluorescence stain, 40X.



Photo courtesy of Tom Larsen



Summary

- WEEV can be lethal by aerosol for both cynomolgus and rhesus macaques.
- Fever onset after aerosol exposure to WEEV is delayed compared to epizootic VEEV-IA/B, similar to what was seen with VEE-IIIa.
- Clinical signs of encephalitis are not seen until fever begins to wane.
- Heart rate increases were seen with both rhesus and cynomolgus macaques. ECG analysis suggests sinus tachycardia, an increase in heart rate commonly associated with fever.
- Infection of the Purkinje cells and hypothalamus suggest direct effect by WEEV on muscle control, including heart rate.
- Pathology also shows massive cellular infiltrate into the CNS, consistent with what has been reported for fatal alphavirus infections in humans.



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